1st WHO International Standard for mycoplasma DNA for nucleic acid amplification technique-based assays designed for generic mycoplasma detection
PEI code 8293/13
(Version 2.0, 04th November 2013)

1. INTENDED USE

This 1st World Health Organization (WHO) International Standard (IS) for mycoplasma DNA (8293/13) is intended to be used in the standardization of nucleic acid amplification technique (NAT)-based assays of generic design, allowing the simultaneous detection of distantly related mycoplasma species. The need to develop a standard was demonstrated in previous studies (1,2) and in the feasibility study of this standardization project (3). This WHO IS was generated from *Mycoplasma fermentans* (strain PG18^T, NCTC 10117).
Mycoplasma fermentans is a bacterial species which may infect humans (without clear pathogenic potential) and which has been described as one of the more frequent mycoplasma contaminants of eukaryotic cell cultures. Mycoplasma contamination of cell cultures may alter cellular parameters, and therefore mycoplasma-free cell cultures are required both in research and in manufacturing environments. In different regions of the world, regulations are in place to assure mycoplasma-free cell cultures used for the manufacture of biomedicinal products. Culture-based approaches of mycoplasma testing have been supplemented or replaced by NAT tests designed for generic detection of mycoplasma species (4).
The project for the "1st World Health Organization International Standard for mycoplasma DNA for nucleic acid amplification technique (NAT)-based assays designed for generic mycoplasma detection" was endorsed by the Expert Committee on Biological Standardization (ECBS) of the WHO in October 2010. The collaborative study performed for the establishment of the WHO IS consisted of two parts: a feasibility study was followed by a comparability study. In the feasibility study NAT assays of different design and of worldwide representation were evaluated with different mycoplasma species. In this study harmonisation of assays (reduction of interassay variation) was demonstrated and the candidate species for the WHO IS was selected. The comparability study confirmed the suitability of the candidate preparation and enabled its assignment of International Units. Further details of the collaborative study are available in the report WHO/BS/2013.2222 (3). The ECBS established this WHO IS in October 2013.

2. UNITAGE

This reagent has been assigned a unitage of 200,000 International Units/ml (2 x 10^5 IU/ml).

3. CONTENTS

Each vial contains 0.5 ml of lyophilized *Mycoplasma fermentans* in Mycosafe Friis culture medium. The bacteria were harvested during the exponential growth phase.

4. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.
The preparation contains lyophilized bacteria which may be potentially infectious and pathogenic for humans. The reference material has been grown in Mycosafe Friis medium tested negative for mycoplasma DNA prior to its use.
As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

5. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

The material is supplied lyophilized and should be stored in the dark at or below -20ºC.

The WHO IS should be reconstituted with 0.5 ml of sterile nuclease-free water.

If all the material is not used immediately, laboratories may aliquot the remaining material into suitable volumes which should be stored at or below -70ºC.
In the collaborative study (3) the WHO IS candidate was characterized with using the routine test matrixes of the laboratories as diluents. These diluents included isotonic buffers, saline, culture medium, cultured cells, cell culture supernatant or virus bulk harvest, without detrimental effects on mycoplasma NAT detection observed.

6. STABILITY

As the stability studies with accelerated conditions indicate high stability of the lyophilized reference material under the recommended storage conditions (≤-20ºC), there is no expiry date assigned to the International Standard. This approach complies with the recommendations for the preparation, characterization and establishment of international and other biological reference standards (5). The International Standard is held at the Paul-Ehrlich-Institut (PEI) within assured, temperature-controlled storage facilities. During its life cycle the stability is monitored at regular intervals. The International Standard remains valid with the assigned potency and status until withdrawn or amended.

Reference materials should be stored on receipt as indicated on the label. Once, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact PEI.

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Email: whoccivd@pei.de
Web: http://www.pei.de

7. REFERENCES

(1) Milne C, Daas A. Establishment of European Pharmacopeia Mycoplasma Reference Strains. Pharmeuropa Bio 2006, 1:57-71.
(2) Dabrazhynetskaya A, Volokhov DV, Lin T-L et al Collaborative Study Report: Evaluation of the ATCC experimental mycoplasma strains reference panel prepared for comparison of NAT-based and conventional mycoplasma detection methods. Biologicals 2013, dx.doi.org/10.1016/j.biologicals.2013.07.002
(3) Hanschmann KM, Montag-Lessing T, Baylis SA et al (2013) Collaborative Study to Establish the 1st World Health Organization International Standard for Mycoplasma DNA for Nucleic Acid Amplification Technique (NAT)-Based Assays. WHO Report 2013, WHO/BS/2013.2222
(4) Volokhov D, Graham LJ, Brorson KA et al Mycoplasma testing of cell substrates and biologics: Review of alternative non-microbiological techniques. Molecular and Cellular Probes 2011, 25:69-77.
(5) Recommendations for the preparation, characterization and establishment of international and other biological reference standards. W HO Expert Committee on Biological Standardization. Fifty-fifth report, 2004. (WHO Technical Report Series, No. 932).

8. FURTHER INFORMATION

About this material: whoccivd@pei.de
About WHO Biological Reference Preparations: http://www.who.int/biologicals/en/

9. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to whoccivd@pei.de

10. CITATION

In any circumstance where the recipient publishes a reference to any WHO IS provided by the WHO Collaborating Centre PEI, it is important that the title of the preparation and the PEI code number, and the name and address of PEI are cited correctly.

11. MATERIAL SAFETY SHEET

Physical properties (at room temperature)		
Physical appearance	Lyophilized powder	
Fire hazard	None	
Chemical properties		
Stable	Yes	Corrosive:No
Hygroscopic	No	Oxidising:No
Flammable	No	Irritant: No
Other (specify) CONTAINS MYCOSAFE FRIIS MEDIUM AND INFECTIOUS MYCOPLASMA FERMENTANS		
Handling:	See caution, section 4	

Toxicological properties
Effects of inhalation: Avoid – *contains infectious Mycoplasma fermentans*
Effects of ingestion: Avoid – *contains infectious Mycoplasma fermentans*
Effects of skin absorption: Avoid – *contains infectious Mycoplasma fermentans*
Suggested First Aid
Inhalation Seek medical advice - *contains infectious Mycoplasma fermentans*
Ingestion Seek medical advice - *contains infectious Mycoplasma fermentans*
Contact with eyes Wash thoroughly with water; seek medical advice – *contains infectious Mycoplasma fermentans*
Contact with skin Wash thoroughly with water; seek medical advice – *contains infectious Mycoplasma fermentans*
Action on Spillage and Method of Disposal
Spillage of vial contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.

12. LIABILITY AND LOSS

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.
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